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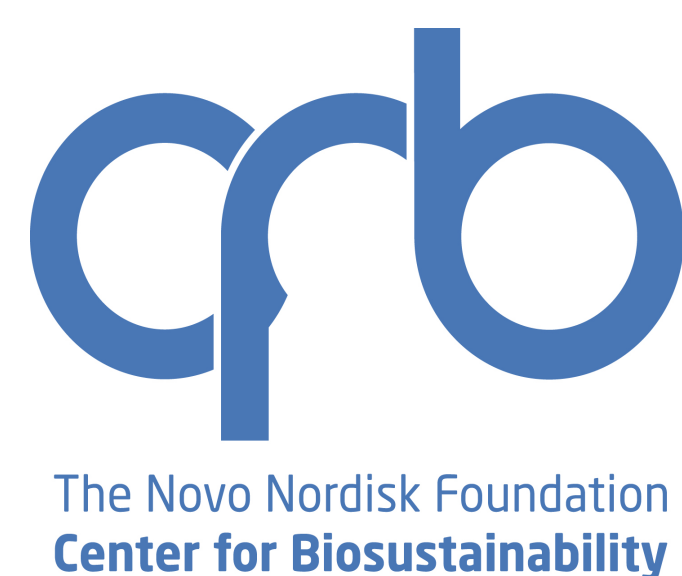
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CHO-glyco-engineering using CRISPR/Cas9 multiplexing for protein production with homogeneous *N*-glycan profiles

PO315

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1. KEY MESSAGE

Combining the chinese hamster ovary (CHO) - K1 draft genome^{1,2}, identified CHO glycosyltransferases³ and the power of multiplexing gene knock-outs with CRISPR/Cas9⁴ via co-transfection of Cas9 and one single guiding RNA (sgRNA) per target, we generated 20 Rituximab expressing CHO-S cell lines differing in amount and combination of insertions or deletions (indels) in the targeted genes. Clones harboring 9, 6 and 4 indels were further investigated for growth, Rituximab productivity and secretome *N*-glycosylation.

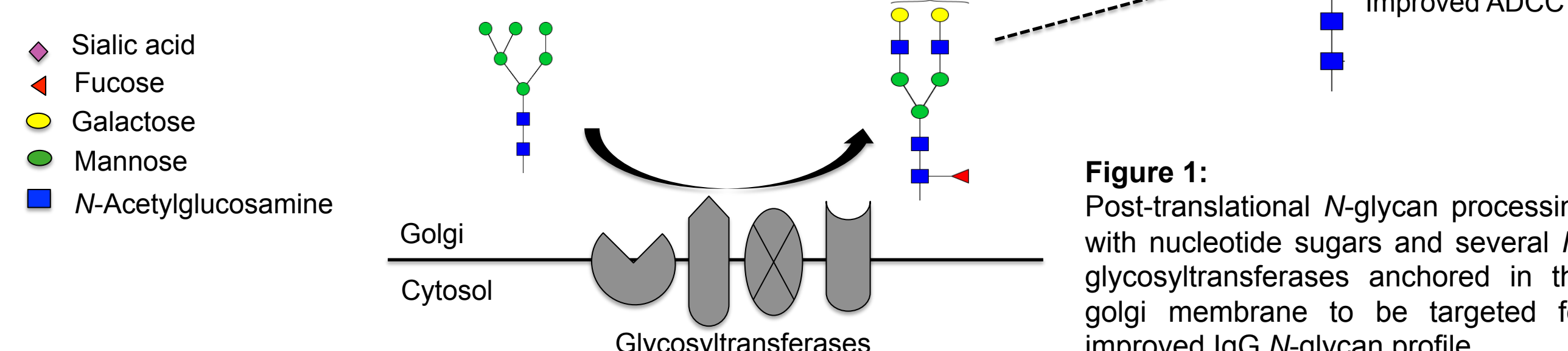
This resulted in clones with prolonged viabilities, no changes in *N*-glycan galactose contents but an increase of matured and sialylated *N*-glycan structures in the secretome. Additionally we point out, that multiplexing an increasing amount of genes most likely results in clones only revealing a few of all possible combinations of the targets and is highly driven by the sgRNA efficiency which can differ from each other by factor 4, even after FACS sorting.

2. Introduction: *N*-glycan engineering

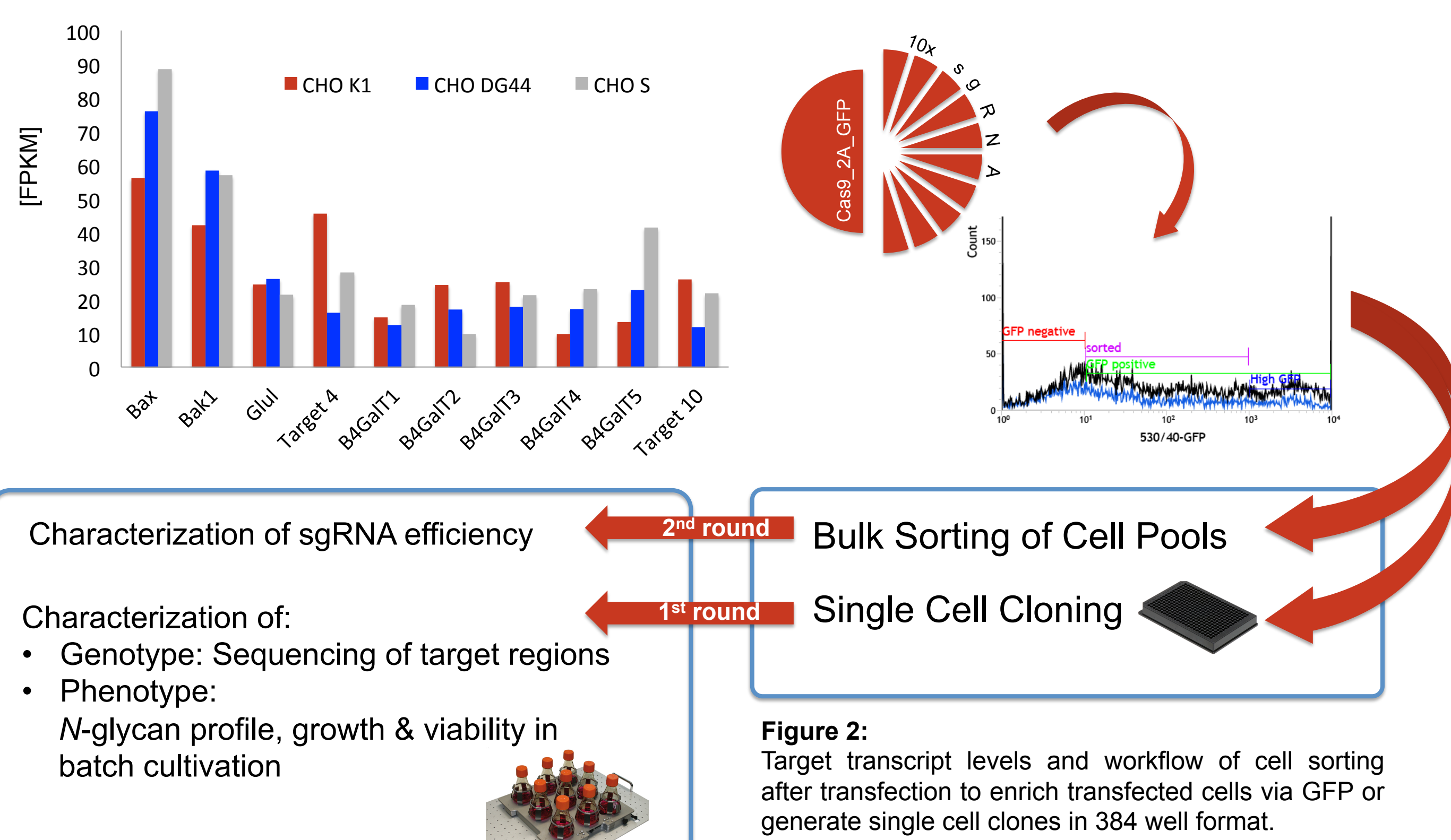
A. Background information

Although CHO cells' strength is the production of similar *N*-glycans to what is found on glycosylated human proteins⁵, non-engineered CHO cells display a broad variety of *N*-glycans which often includes *N*-glycan structures, that have an undesired effect on e.g. efficacy, antibody-dependent cell cytotoxicity (ADCC) or lectin-mediated clearance of the glycoprotein. In this work, we investigate the limitations of targeting up to **ten gene targets** via multiplexing in a Rituximab producing CHO cell line. The targets include *N*-glycosyltransferases, enzymes involved in nucleotide sugar synthesis, *N*-glycosyltransferase modulation, apoptosis and glutamine synthesis.

B. Hypothesis

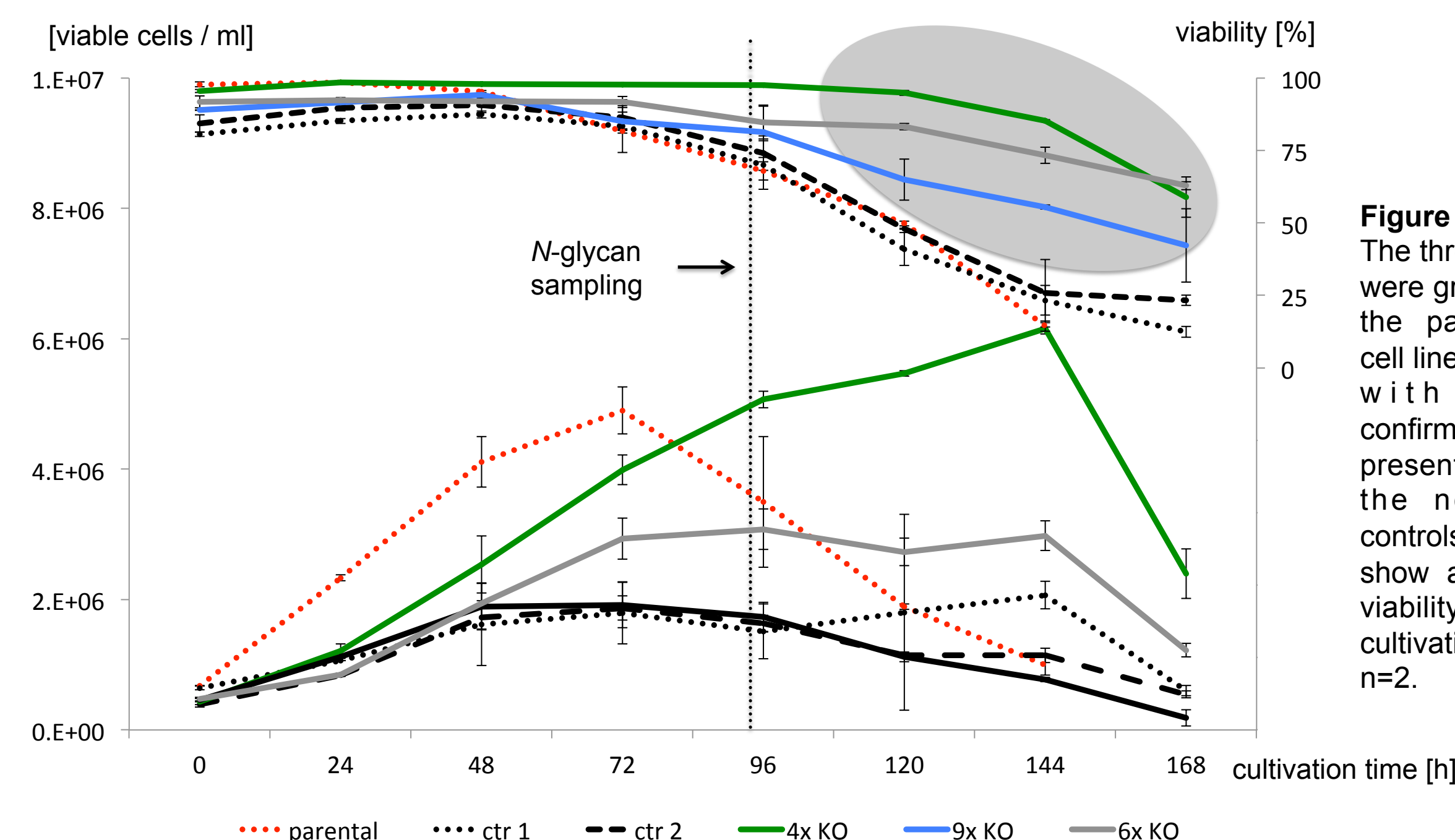


3. Experimental Overview

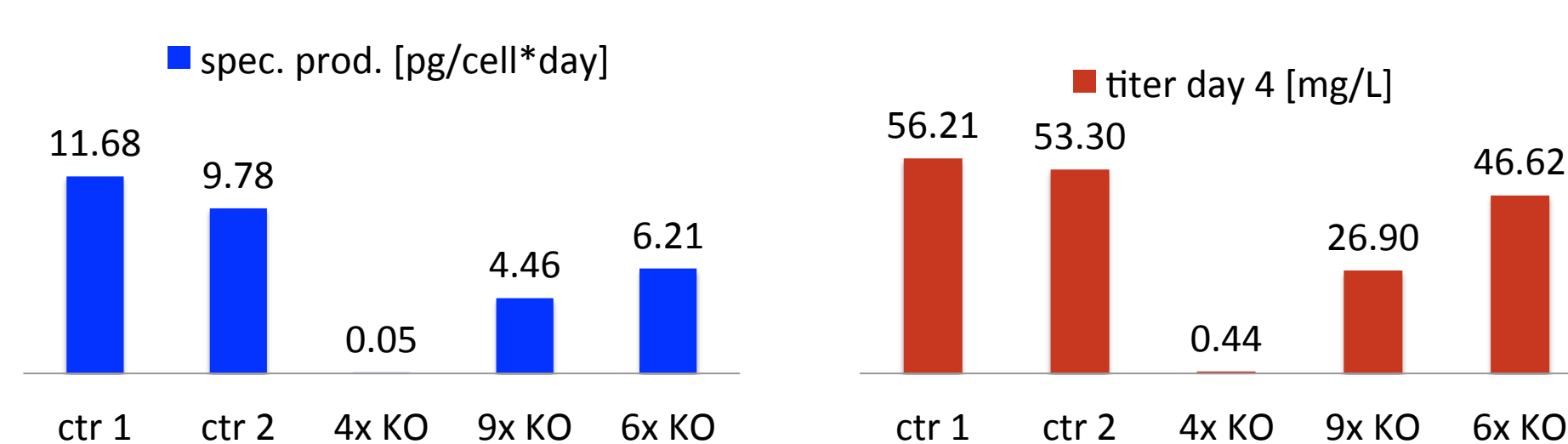


5. Results: Growth, Rituximab titers and secretome *N*-glycosylation

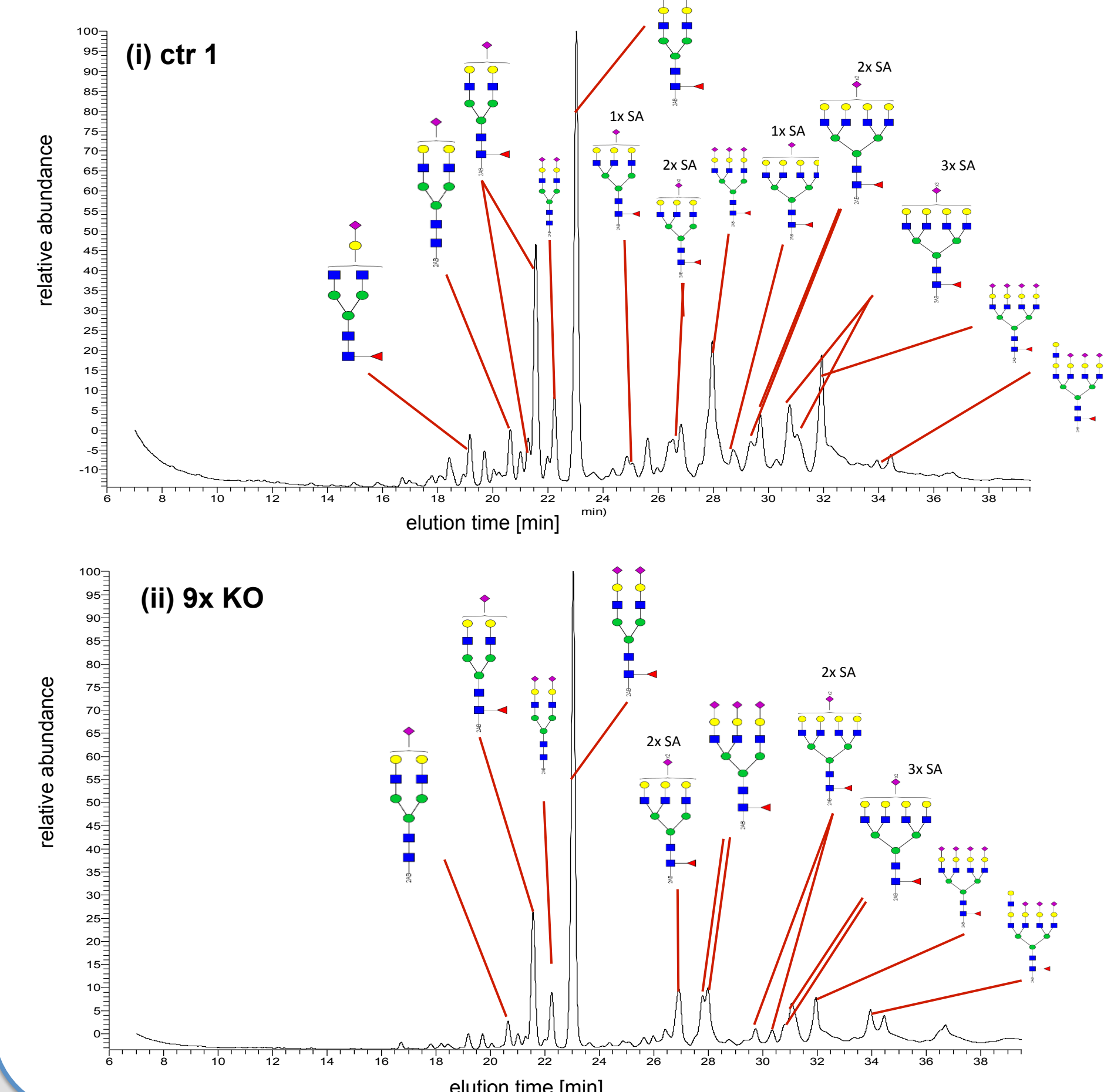
A. Growth and Viability in Batch Experiment



B. Rituximab quantification



C. Secretome *N*-glycan analysis



4. Sequencing of pools and clones

A. Clone Level Sequencing

Target	Bax	Bak1	GLUL	Target 4	B4GalT1	B4GalT2	B4GalT3	B4GalT4	B4GalT5	Target 10
9x KO										
6x KO										
4x KO										
5x A										
4x A										
4x B										
3x A										
3x B										
3x C										
2x A										
2x B										
2x C										
2x D										
1x A										

Legend: Indel partially in-frame (hatched), Indel out of frame (black), WT sequence (white), Phenotype based GLUL-KO (no growth in glutamine-free medium) (red), batch in 30 ml shake flask for growth, titer and *N*-glycan analysis (blue).

Figure 3: Out of 94 screened clones, 20 clones harbor at least one genetic modification after multiplexing with 10 sgRNA's (top 14 clones shown here).

B. Pool Level Sequencing

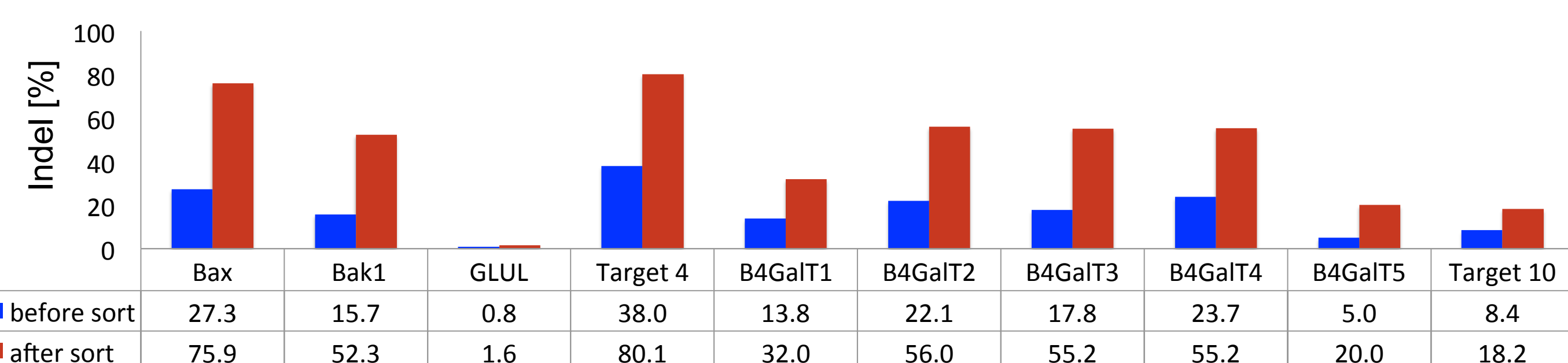


Figure 4: Frequency of indel generation after pool level sequencing of the different target regions before (blue) and after (red) FACS sorting for GFP-positive cells representing Cas9_2A_GFP expressing populations. The frequency of indel generation increased at least one-fold for all sgRNA targets after FACS and range from 1-80%.

- References:
- Xu, X. et al. Nat. Biotechnol. 29,735–741(2011)
 - Lewis, N.E. et al. Nat. Biotechnol. 31,759–765(2013)
 - Yang, Z. et al. Nat. Biotechnol. 33, 2014–2017 (2015)
 - Grav LM et al. Biotechnol. J. 10 (9), 1446–1456 (2015)
 - Walsh, G. Drug Discov. Today 15,773–780(2010)



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